Chapter 2

Incorporation of Primary Amines via Plasma Technology on Biomaterials

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Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/59691

1. Introduction

1.1. Tissue engineering

A shortage of organs and tissues for transplantation has been present throughout most of the history of transplantation. Over the past few decades, the increasing incidence of vital organ failure and the severe shortage of donors have created a wide gap between organ supply and organ demand, which resulted in very long waiting times to receive an organ as well as an increasing number of deaths while waiting. Moreover, all manners of projections indicate that this gap will continue to widen making this a main challenge to modern medicine [1, 2].

Over the years and in order to overcome many challenges in the area of healthcare, technological advancements rapidly evolved and became a crucial part of modern medicine by helping ensure a better lifestyle and an increased life expectancy. Hence, the field of tissue engineering (TE) emerged in response to that growing need for tissues and organs for transplantation and has rapidly become one of the most exciting advances in regenerative medicine. TE is a multidisciplinary field combining principles of biology, medicine and engineering that aim at generating completely biocompatible fully functional organs or tissues that could be used to replace damaged or missing tissues in reconstructive surgery [3, 4]. The numerous and complex problems arising when replacing tissues set very high and diverse requirements on the used materials: biodegradability, enabling cell attachment and proliferation and mechanical strength are some of the possible demands.

It is very difficult to find an adequate material that meets all requirements to function properly in a bio-environment. A way is to select a material having the required bulk properties such as mechanical strength and sometimes biodegradability and modify its surface properties via a surface treatment.

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The success of an implant is determined by the response of its surrounding biological environment. This is governed to a large extent by the surface properties of the biomaterial where the interaction happens. Correspondingly, considerable efforts have been focused on surface engineering of biomaterials in order to give them the ability to promote cell adhesion, proliferation and to maintain cell functions. Therefore, a clear characterization of the physical and chemical properties of the biomaterial surface has a major scientific importance on cell-biomaterial interactions allowing the evaluation of the bioactivity of the surface engineered biomaterial.

Up to present, a large number of surface engineering techniques for improving biocompatibility have been well established. The work generally contains three main steps: after the surface modification of the biomaterial is done, chemical and physical characterizations are conducted followed by a biocompatibility assessment through in vitro cell culture [5, 6].

In the past decades, surface treatment of biomaterials with plasma has been extensively studied [7-9]. Plasma modification of biomaterials gives the opportunity to change the surface characteristics to achieve better biocompatibility without altering the bulk properties. At the same time, plasma surface modification is a very versatile technology: the results of plasma modification can be easily controlled by choosing suitable gases or monomers and the appropriate conditions in the plasma [10].

1.2. Plasma

In order to introduce plasma it is often stated that plasma is the fourth state of matter in the sequence: solid, liquid, gas, and plasma. The state of matter changes from solid to liquid to gas to plasma by increasing the temperature of the material under consideration (see figure 1).

When gas atoms are subjected to energy (thermal, electrical or light) they become ions by releasing some of their electrons. Collisions between electrons and molecules and bond breaks in molecules create radicals. Energy will also create excited species that will generate photons. This is how plasma is created with a unique mixture of electrons, ions, radicals, photons and neutral atoms and molecules [11, 12].

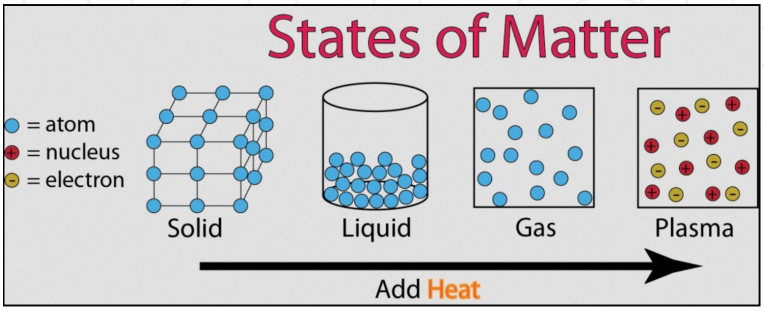

Figure 1. States of matter [90]

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Based on the relative temperatures of electrons, ions and neutrals, plasmas are classified as thermal "equilibrium" and non-thermal "non-equilibrium". Due to the light mass of the electrons present in the plasma, these electrons are instantly accelerated by the electric field to higher velocities than the heavier ions in the time available between collisions. When the collision occurs only a small fraction of the electron energy is lost. This is why the electron temperature in the plasma is initially higher than that of heavy particles. The resulting plasma is a non-thermal or cold plasma in which the electron temperature (≈ 10000 °C) is much higher than the gas temperature (< 200 °C). However, if the pressure is so high that the charged particles do not move very far before the next collision or the electrical field is very low, the energy of the electrons may tend towards that of the heavy particles. In this case, the resulting plasma is a thermal or hot plasma.

Plasmas used in "plasma technology" are usually cold plasmas in the sense that only a small fraction of the gas molecules are ionized which can provide electrons and ions at the right energy without excessive heat enabling the use of plasma on heat-sensitive materials such as polymers [13]. The most commonly used method for generating and sustaining a low-temperature plasma for technological and technical applications is applying an electric field to a pure or mixed gas.

Different plasma sources are available and their dimensions are determined largely by the particular application for which the plasma is intended. There are distinct differences not only in the physical shape of various plasma sources, but also in the temporal behavior of the plasmas that are generated in different sources.

2. Plasma generation

2.1. Plasma sources

As already mentioned, for surface modification treatments, various plasma sources are available. Each of them has its own characteristics such as density, temperature, chemical composition, etc., and leads to different results. The choice of the proper source for the specific task requires the study of the characteristics of the various plasmas. In this section, a brief summary of the most common plasma sources used for the incorporation of amine functional groups in tissue engineering will be given.

2.1.1. Microwave (MW) plasma

MW discharges are electrical discharges generated by electromagnetic waves with frequencies between 300 MHz and 10 GHz. MW discharges represent a simple way of plasma generation both with high (> 100 W/cm^3) and low (< 1 W/cm^3) power levels and can be used over a wide region of operating pressures (from 10^{-3} Pa up to atmospheric pressure). Nowadays, these discharges are widely used for generation of quasi-equilibrium and non-equilibrium plasmas for different applications because of the simplicity of control of the plasma internal structure by means of changes of the plasma characteristics and the possibility of plasma generation

both in small and large chambers. The plasma absorbed power can be high enough and runs up to 90% of the incident power [14, 15].

2.1.2. Radiofrequency (RF) plasma

RF discharges usually operate in the frequency range f=1–100 MHz. The power coupling in RF discharges can be accomplished in different ways: capacitively coupled discharges and inductively coupled discharges.

- Capacitively coupled plasma (CCP)

CCP is generated with high-frequency RF electric fields, typically 13.56 MHz. In its simplest form, the RF voltage is applied across two parallel metal plates, generating an oscillating electric field between them. This field accelerates electrons leading to an ionization avalanche. The parallel electrodes which are separated by a distance of a few centimeters may be in contact with the discharge or insulated from it by a dielectric. Gas pressures are typically in the range 1–10^3 Pa. In a capacitively coupled RF discharge, the electron density is in the range n_e=10^9-10^{10} cm^{-3} and densities up to 10^{11} cm^{-3} are possible at higher frequencies.

- Inductively coupled plasma (ICP)

ICP is similar to CCP but the electrode consists of a coil wrapped around the discharge volume that inductively excites the plasma. ICP is excited by an electric field generated by a transformer from an RF current in a conductor. The changing magnetic field of this conductor induces an electric field in which the plasma electrons are accelerated. ICPs can achieve high electron densities (n_e=10^{12} cm^{-3}) at low ion energies [15, 16].

2.1.3. Dielectric Barrier Discharge (DBD)

Dielectric barrier discharges (silent discharges) are non-equilibrium discharges that can be conveniently operated over a wide temperature and pressure range. DBDs are characterized by the presence of one or more insulating layers in the current path between metal electrodes in addition to the discharge space. At a sufficient AC voltage, electrical breakdown occurs in many independent thin current filaments. These short-lived microdischarges have properties of transient high pressure glow discharges with electron energies ideally suited for exciting or dissociating background gas atoms and molecules.

Due to charge build up on the dielectric, the field at the location of a microdischarge is reduced within a few nanoseconds after breakdown thus terminating the current flow at this location. The current density in a microdischarge channel can reach 100 to 1000 Acm^{-2}. Due to the short duration, this normally results in very little transient gas heating in the remaining channel. The dielectric barrier limits the amount of charge and energy deposited in a microdischarge and distributes the microdischarges over the entire electrode surface. As long as the external voltage is rising, additional microdischarges will occur at new positions because the presence of residual charges on the dielectric has reduced the electric fields at positions where microdischarges have already occurred. When the voltage is reversed, however, the next microdischarges will form in the old microdischarge locations.

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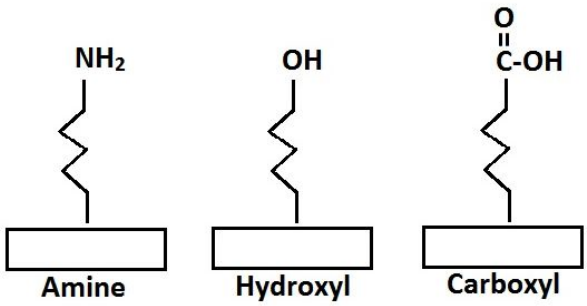

Figure 2. Primary amine, hydroxyl and carboxyl functionalities

Its flexibility with respect to geometrical configuration, operating medium and operating parameters is unprecedented. Conditions optimized in laboratory experiments can easily be scaled up to large industrial installations.

Although DBD configurations can be operated between line frequency and microwave frequencies the typical operating range for most technical DBD applications lies between 500 Hz and 500 kHz [17].

Plasma can be used in the continuous wave (CW) or pulsed mode. In the continuous wave mode, plasma with a specific power is turned on for a specific amount of time. In the pulsed mode, plasma is intermittently generated with a fixed duty cycle (Δ).

$$\Delta = t_{on} / \left(t_{on} + t_{off} \right) \tag{1}$$

where t_{on} is the time during which the plasma is turned on and t_{off} is the time during which the plasma is turned off. The mean power (P_{mean}) is then defined by equation (2) and represents the average energy dissipated in the plasma period, with P_{peak} the power injected during t_{on}[18].

$$P_{mean} = \Delta * P_{peak} \tag{2}$$

2.2. Plasma media

Among other physical and chemical techniques to improve the surface biocompatibility [19-22], plasma surface modification is used to adapt the surface properties by functionalization of the material surface to control the biological response. Different functionalities have been investigated, such as carboxyl [23], hydroxyl [24] and primary amine [25] (see figure 2). In this chapter, we will focus on plasma surface modification of biomaterials by means of primary amine group incorporation.

Amino groups are usually incorporated on the surface using either non-polymer-forming gases (amine plasma activation) such as ammonia (NH_3) or nitrogen (N_2), or amine monomers (amine plasma polymerization) in the plasma medium. The former etches the biomaterial surface and introduces nitrogen functionalities; the latter deposits a plasma polymer layer containing nitrogen groups on the surface [26].

Plasma polymerization of amine-based monomers is an efficient way to prepare bioactive amino functionalized polymer surfaces. Amine-functionalized surfaces have previously been obtained through plasma polymerization using different monomers such as allylamine [27, 28], ethylenediamine [29-31], n-heptylamine [32, 33], propylamine [34, 35], cyclopropylamine [18], diaminocyclohexane [36, 37], and butylamine [26].

In the biomedical field, the most common used plasma media are ammonia as non-polymer forming precursor and allylamine as polymer forming precursor.

Primary amine ($-NH_2$) functional groups can promote covalent immobilization with biomolecules such as protein like antibodies, collagen and DNA [28, 38]. Moreover, protonated amines can introduce a localized positive charge in aqueous solution at physiological pH value, which can potentially be used for electrostatic interactions with negatively charged cells and proteins and is propitious to promote cell adhesion and proliferation [39, 40].

In this chapter we will focus more on amine plasma polymerization since this is the most commonly used plasma technique for the incorporation of primary amines. Plasma polymerization has unique practical advantages which include (i) ultra-thin film deposition, (ii) good adhesion to the substrate material, and (iii) chemically stable and physically durable nature of the polymers [38].

3. Characterization techniques

Since the surface of an implant will be readily in contact with the biological environment, the surface characteristics of a biomaterial will have a major influence on the cell-material interactions. These characteristics should be analyzed and studied in order to correlate them with the material biocompatibility and thus try to improve it by changing them.

In order to evaluate the effect of plasma treatments on the surfaces, physical and chemical characterizations are performed using different techniques, such as: water contact angle measurement (WCA), Fourier-transform infrared (FTIR) spectroscopy, X-ray photoelectron spectroscopy (XPS), atomic force microscopy (AFM) and scanning electron microscopy (SEM). In this section, a brief definition of each of these techniques and their correlation with amine plasma treatments will be given.

3.1. Water Contact Angle measurements (WCA)

WCA analysis is a simple and widely used test to evaluate the wettability of a surface by measuring the static contact angle of small droplets of distilled water or other liquids on the

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surface. The contact angle of a material is where a liquid/vapour interface meets a solid surface. It quantifies the wettability of a solid surface and thus the relative amounts of adhesive (liquid-to-solid) and cohesive (liquid-to-liquid) forces acting on a liquid [41].

If the WCA is smaller than 90°, the solid surface is considered hydrophilic and if it is larger than 90°, the solid surface is considered hydrophobic (see figure 3) [42].

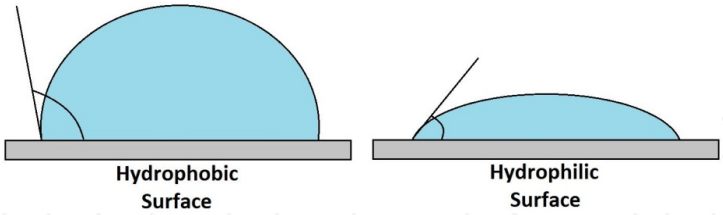

Figure 3. Principle of contact angle measurement

Amine plasma treatment causes a decrease in WCA due to the incorporation of hydrophilic nitrogen and oxygen moieties [43-45] onto the sample surface. Oxygen functionalities can be incorporated via two different processes. First, the vacuum level does not guarantee total absence of oxygen impurities in the plasma chamber [46, 47]. Second, after plasma treatment, samples are exposed to air which can induce a post plasma functionalization by reaction with oxygen and water vapor [47, 48].

Additionally, depending on the gas and general conditions of the plasma treatment, it is possible to promote some surface etching/abrasion which can induce changes in surface topography, thus having a certain effect on the wettability. Material removal occurring during plasma treatment increases roughness and contributes to improve the wettability properties of the plasma treated film [44, 49].

3.2. X-ray Photoelectron Spectroscopy (XPS)

X-ray photoelectron spectroscopy is a very powerful surface analysis technique also known as electron spectroscopy for chemical analysis (ESCA). It works by irradiating a sample with mono-energetic soft X-rays causing surface electrons to be ejected. The identification of the elements in the sample can be made directly from the kinetic energies of these ejected photoelectrons, and the relative concentrations of elements can be determined from the photoelectron intensities.

An important advantage of XPS is its ability to obtain information on chemical states from the variations in binding energies, or chemical shifts, of the photoelectrons [50].

Since a wide range of chemical functionalities is introduced on the surface of a plasma treated material [43], each of these groups might cause the observed binding energy of a particular peak (e.g. C 1s or N 1s) to shift to varying degrees. The resulting overall peak shape is a superposition of many components which are not clearly resolved.

Curve-fitting can be a powerful method of extracting additional information from XPS data in which contributions from different chemical species can be quantified.

A high amount in nitrogen atoms does not necessary impart a high amount of amine groups on the polymer surface. Peak fitting of the high-resolution spectrum of the N 1s peak confirms the addition of different nitrogen functionalities to the surface during plasma treatment. The assignments of the different nitrogen groups under the N 1s peak found in literature are: amines (398.9-399.3 eV) [32, 51, 52], nitriles (399.6 eV) [53], amides (399.8 eV) [32, 51-53], imides (400.5 eV) [32, 51-53] and quaternary amines (401.3-401.5 eV) [52-54]. Moreover, in the peak fitting of C 1s in amine plasma deposited films two additional peaks might be observed: 286.4 eV corresponding to C-N (amine) and 288.0 eV corresponding to N-C=O (amide) groups [45, 55]. The areas under the photoelectron peaks in the spectrum are used to calculate the atomic concentrations.

Another more meaningful identification and quantification method of the present chemical groups is a combination of XPS with chemical derivatization. Chemical derivatization consists in inducing a chemical reaction between a targeted chemical group of the amine plasma polymer film and a chemical reactant containing at least one atom different from the ones composing the sample. For a successful derivatization process, selectivity of the reagent towards a particular functional group, its detectivity, kinetics of the reaction, and stability of the derivatized species are some of the factors necessary to identify [55].

In the case of amine plasma treatments, to selectively probe $–NH_2$ groups, several reactants such as 4-trifluoromethyl-benzaldehyde (TFBA) [18, 36, 39, 55-60], pentafluoro-benzaldehyde (PFBA) [61, 62], and para-chlorobenzaldehyde [63] have been used.

The derivatization reaction performed by exposing the treated sample to TFBA vapor is the most commonly used and it consists in a nucleophilic addition on the carbonyl group (C=O) that converts an NH_2 group into an imine (see figure 4). Hence, NH_2 groups are selectively probed by the reagent CF_3 terminal group with a ratio of one CF_3 for one NH_2. After the derivatization step, the $\%NH_2$ is calculated from XPS according to [56]:

$$\%NH2 = \frac{[NH_2]}{[N]} = \frac{([F]/3)}{[N]} . 100\% \quad (3)$$

where $[NH_2]$, [N] and [F] represent respectively the relative concentration of primary amines, nitrogen and fluorine at the sample surface.

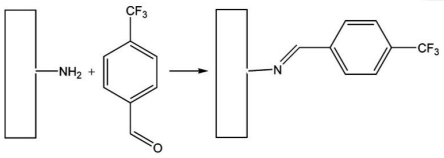

Figure 4. Reaction scheme for TFBA derivatization of primary amine functionalities [91]

3.3. Fourier-Transform Infrared (FTIR) spectroscopy

FTIR is based on the interaction of an oscillating electromagnetic field with a molecule. In a specific compound, a particular structural group reveals IR absorption bands within characteristic spectral regions. In this way, FTIR can be used for assignation of functional groups and identification of pure compounds.

During plasma polymerization, the monomer undergoes reorganization due to the breakage and the recombination of bonds. Amine groups are partially transformed into amide, imine or nitrile functional groups [36, 64]. In various allylamine plasma polymerization studies, one can see after comparison with the pristine monomer that some bands are significantly broadened, some disappeared while new bands also appeared. An example of these spectra can be seen in figure 5. Double peaks of primary amine N-H stretching vibrations at 3380-3290 cm^{-1} are well resolved on the spectra of the monomer but a wide absorption band is found on the polymer at 3390 cm^{-1} which can originate from a primary amine, a secondary amine or an imine as well. The deformation vibration of primary amines (1510-1650 cm^{-1}) is observed in both spectra, but is considerably broadened in the spectrum of plasma polymerized allylamine (PPAa), which indicates the presence of alkene groups or imines. A new band appears for the polymer at 2200 cm^{-1} which is associated with the stretching vibration of nitrile (C≡N) groups [43, 64, 65].

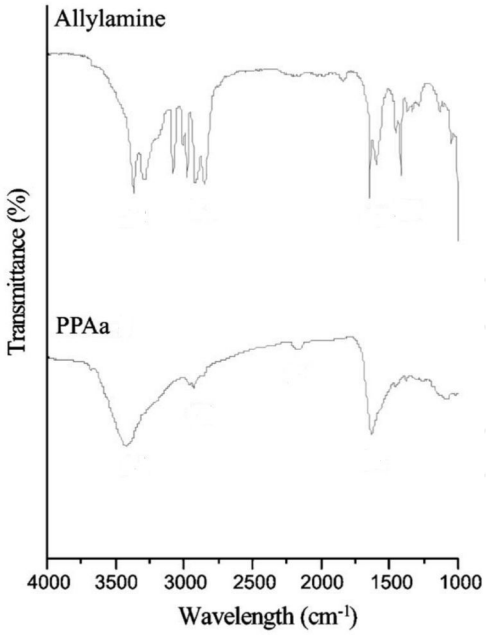

Figure 5. FTIR spectra of allylamine and PPAa [92]

3.4. Scanning Electron Microscopy (SEM)

SEM images the sample surface by scanning it with a high-energy beam of electrons. Depending on the instrument, the resolution can fall somewhere between less than 1 nm and 20 nm.

SEM has been used in different studies involving plasma treatments. For instance, in a study by Hamerli et al. [43], SEM images showed that allylamine plasma polymerization yields homogenous pinhole-free layers. In another study by Sanchis et al. [44], SEM images showed that nitrogen plasma treatment formed micro-cracks on the sample surface. In this way, SEM can be used on plasma treated samples to give information regarding the deposited film morphology and the treatment effect on the sample surface.

3.5. Atomic Force Microscopy (AFM)

AFM is a mechanical imaging instrument that measures the three-dimensional topography as well as physical properties of a surface with a sharpened probe. Typical AFM resolutions are well below 1 nm.

AFM can be used to study the way plasma polymers grow: AFM images have been used to show the surface morphology of the deposited films while varying the treatment time [66]. AFM analysis is useful, not only in a qualitative way but also for quantitative determinations, since it allows a 3D representation of the treated surface and quantifies the effects of the plasma-etching mechanism by calculation of the surface roughness (root-mean-squared roughness, R_{rms}) [44].

4. Effect of plasma process parameters on final layer properties

Many parameters, such as the nature of the precursor, the precursor flow rate, the reactor chamber's pressure, the reaction time, the power and frequency of the discharge, the geometry and physical dimensions of the reactor, etc. are believed to influence the surface properties of the resulting thin film [67].

In this section, a summary of the effect of plasma process parameters on various amine-deposited film properties as elucidated by researchers in this field will be given. Process parameters will be discussed based on different studies, but one should always keep in mind that various results emerging from different studies are hinted by the possible role of the distinctive processes (different reactor, plasma media and plasma source) used in each case.

4.1. Discharge power and deposition time effect

Electrical power and treatment time have a large impact on the nature and concentration of the molecular species formed in the reactor, which in turn significantly influences the deposition rate and the final atomic composition of the deposited layers.

Effect on deposition rate / film thickness: Lucas et al. [61] studied the power variation (between 3 and 100 W) effect on the deposition rate of allylamine plasma polymers. They found that

deposition rate increases with increasing power. The same effect was observed by Myung et al. [64] with power variation between 30 and 90 W. The thickness of the film increases with increasing plasma power since high power generates high plasma density which increases the deposition efficiency. However, in another study by Lejeune et al. [57], the increase of deposition rate with power was found to be valid only up to a certain limit. In fact, the deposition of a film relies on a dynamic equilibrium between a process of deposition and a process of sputtering by incoming particles from the plasma. At low power of deposition ($P < P_{limit}$), the first process dominates the equilibrium. The amount of low energetic particles arriving on the surface of the film increases and the rearrangement of these deposited particles is low. This growth mode favors the formation of a low density structure (low cross-linking degree) with a high growth rate. With the increase of the power, the incoming particles have a higher energy and can penetrate more deeply in the growing film. Structural reorganization processes such as cross-linking of the polymeric chains and re-sputtering phenomena can occur. When the power is high enough ($P > P_{limit}$), the second process of the dynamic equilibrium acts effectively on the deposition: due to the sputtering effect, the growth rate becomes constant and due to the bombardment effect, the density of the film increases (high cross-linking degree) (see figure 6). For Lejeune et al. the limiting power was 30 W, but as already mentioned, results from different sources can only be compared to a certain extent and this limiting power varies from one treatment to another by taking into account the plasma media, plasma source, plasma parameters and reactor geometry.

Myung et al. [64] also investigated the effect of treatment time on the deposition rate. They found that the thickness of the deposited layers increases with the increase of plasma polymerization time. Martin et al. [68] investigated the synergistic effect of plasma power and deposition time on n-heptylamine plasma polymerized (HApp) film thicknesses using AFM step height measurements combined with a surface masking technique. The results showed a dramatic difference between conditions involving high power and long deposition (thickness average of 47 nm) to those involving low power and short deposition time (thickness average of 3 nm) (see figure 7).

Effect on film's atomic composition: Martin et al. [68] also studied the effect of power on the atomic composition of the layers using XPS analysis. High power yielded relatively lower surface concentration of nitrogen atoms than the use of low power: by varying the power from 80 W to 10 W nitrogen content increased by ca. 15%. This is due to the more successful breakdown of the monomer molecule achieved at higher power, yielding layers which present less nitrogen atoms. This behavior has also been observed by Shard et al. [35]. However, deposition time and time-power interaction do not have a significant effect on the atomic composition of the layers [68].

In the study conducted by Lucas et al. [61], the use of XPS coupled to derivatization reactions for allylamine plasma polymerization showed that $\%NH_2$ decreases with the increase of power. This was also observed by Lejeune et al. and Müller et al. [57, 69]. The effect of plasma on the retention of the precursor functional group also depends on the plasma mode. Basarir et al. [70] worked on plasma polymerization of allylamine using both CW and pulsed modes. Results showed that pulsed plasma polymerization further increased amine density. In fact,

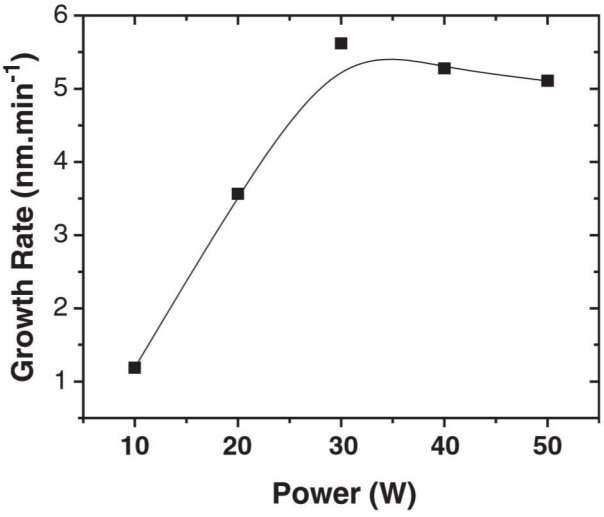

Figure 6. Growth rate of plasma polymerized allylamine films as a function of power [93]

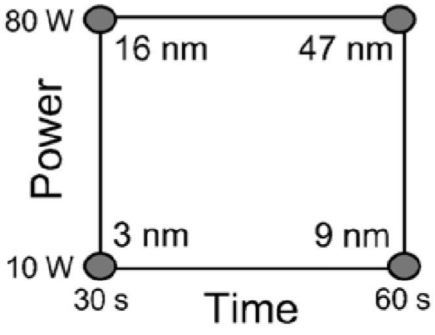

Figure 7. Square plot summarizing the influence of power and deposition time on the thickness of HApp layers [94]

in the pulsed mode, the mean power per precursor molecule is lower than that in the CW mode. In the off-time, dissociated monomers react with each other instead of continuing to dissociate. Moreover, the use of lower P_{mean} in pulsed plasma polymerization results in a higher retention of the primary amine functional group [18, 56].

By using IR spectroscopy on allylamine plasma deposited films, Myung et al. [64] noticed changes in film composition by varying input power between 30 and 90 W. High-power plasma led to a higher ratio of C≡N to CH than the ratio of NH to CH, thus to a recombination of amine functionalities into nitrile (C≡N) groups. This was also observed in two studies by Hamerli et al. [43, 47]. At high power, monomer fragmentation is accelerated and results in the formation of imine groups and nitrile groups. A high retention of amine groups is mainly favored by low input power (< 30 W). This has been confirmed by Wen-Juan et al. [71] where

power was varied between 5 and 30 W. The increase of plasma power caused more formation of C=N groups which can be explained by the sufficient fragmentation of monomer at higher applied powers.

Furthermore, Myung et al. [64] correlated the contact angle to the plasma input power. The contact angle increases with increasing input power thus causing a decrease of the surface free energy i.e. a decrease of the surface hydrophilicity. The main factors which resulted in this effect were the loss of amine functional groups and the formation of cross-linked structures due to more fragmentation of allylamine by increasing input power. In another study by Sanchis et al. [44], treatment time effect on surface morphology was investigated via AFM imaging. Different surface morphologies with a slight increase in surface roughness were observed as exposure time increased.

As we can see, power has a great influence on the chemical and physical properties of the deposited films. The synthesis conditions for deposition of plasma polymer films with high functional group concentration are characterized by low powers thus leading to low precursor fragmentation. This will result in the reduction of the plasma polymer film cross-linking degree [56]. However, the control of the cross-linking degree is an important factor for the optimization of the plasma polymer film stability and mechanical and thermal properties [72]. Therefore, it is important to evaluate the cross-linking degree in addition to the plasma and polymer film chemistry in order to choose the appropriate power [70, 73].

4.2. Monomer flow rate effect

Monomer flow rate is an important plasma process parameter that has been investigated in order to correlate it to the deposited film properties.

Hamerli et al. [43] and Basarir et al. [70] investigated monomer flow effect on amine functionality retention. At constant power and treatment time, higher monomer flow rates yielded higher amine retention. The increased amine density with increased flow rates can be explained by less dissociation of monomers, owing to the decreased plasma power for each molecule as the monomer flow rate increased.

Another study by Martin et al. [68] showed that the monomer flow rate does not influence the thickness of the deposited layers.

4.3. Precursor type effect

The precursor type is one of the most important factors in plasma treatments. For instance, in plasma polymerization, the use of a non-saturated monomer like allylamine is advantageous compared to saturated ones because in the former case less energy is needed for the polymerization process. In fact, the double bond in allylamine encourages a deposition by a combination of plasma and conventional free-radical polymerization. Because of that, allylamine typically polymerizes at low energies. Due to less fragmentation, a higher amount of primary amines can be retained in the plasma polymer [34, 59]. Furthermore, each precursor is characterized by its own chemical structure and thus its particular bond breaking energies

which influence to a great extent the selectivity of the fragmentation processes in the plasma. Therefore, depending on the kind of monomer used, various chemical compositions are obtained after each plasma treatment [18, 25, 55]. For example, Hamerli et al. [47] used ammonia and allylamine as plasma precursors. A higher amine concentration was found on the allylamine modified samples. Mangindaan et al. [60] used allylamine, propylamine and propargylamine (another unsaturated monomer) as precursors. XPS coupled to derivatization showed that allylamine incorporates the highest amount of amine functionalities into the corresponding thin films compared to those synthesized from the two other precursors.

The precursor type also influences the growth mode and thickness of the deposited layers. Michelmore et al. [66] noticed that films grown from n-heptylamine initially show "island-like" growth before a continuous smooth film is formed. In contrast, films from allylamine grow smoothly from the very earliest stages. Moreover, it has been found that monomers containing double bonds polymerize faster in plasma than their saturated counterparts. Gancarz et al. [25] have investigated the plasma polymerization of n-butylamine and allylamine and observed that the deposited layers are much thicker for allylamine plasmas. This observation has also been confirmed in a study performed by Mangindaan et al. [60].

5. Interaction between plasma induced amino groups and cells

In this section, an overview of literature on plasma treatments used for the incorporation of amine functionalities and their influence on cell-material interactions will be given. It is important to note that each precursor compound and each plasma treatment is unique and will yield different surface chemical and physical properties. Hence, for the same plasma media, a different plasma treatment will yield specific surface characteristics. And for the same plasma treatment, a different plasma media will also yield specific surface characteristics. Moreover, a specific type of cell reacts differently with specific surface properties.

Cell adhesion and proliferation on amine plasma polymer films deposited onto different substrates have been widely investigated. Different plasma methods have been used and different cells and biomolecules interactions with the treated surfaces have been investigated for various biomedical applications. In the following, only some of the main studied bio applications will be explored. An overview of the various cited works on cell-material interactions for different amine plasma treatments is given in table 1.

One important studied bio application is hemocompatibility of biomaterials used in blood contacting devices [74]. Hemocompatibility is considered to be one of the most critical aspects of biocompatibility. In order to achieve better hemocompatibility, a common approach is to immobilize heparin on the surface of the implant. Heparin is a strongly acidic, negatively charged polysaccharide (see figure 8) used in preventing thromboembolic complications due to its high affinity binding to antithrombin III (ATIII) resulting in its conformational change thus accelerating its ability to inactivate the coagulation enzymes [75, 76]. Metallic biomaterials used for vascular devices have excellent mechanical properties and corrosion resistance but have insufficient long-term hemocompatibility. Yang et al. [77] modified stainless steel using

radio frequency plasma and a mixed gas of allylamine vapor and NH_3. After plasma treatment, an FTIR and XPS study of the deposited film showed a good retention of the allylamine structure and thus the presence of primary amine functionalities on the surface. Nevertheless, the appearance of some new peaks in the spectra of the deposited film suggests that the primary amine groups are partially transformed into nitrile, amide or imine functional groups. As a consequence, the hydrophilicity was greatly improved. The water contact angle decreased from 70.8 to 62.7°. Hemocompatibility improvement was successfully achieved by heparin immobilization on the plasma polymerized allylamine. Cell culture tests were also conducted using endothelial cells which were found to adhere and proliferate in a better way on the plasma polymerized allylamine coating. Both improved hemocompatibility, adhesion and proliferation of endothelial cells were attributed to a combined effect of increased wettability and amine based surface chemistry. In another study [78], the improvement of hemocompatibility of polymeric vascular prosthesis such as polytetrafluoroethylene (PTFE), polystyrene and silicon was investigated. After depositing diamond-like carbon (DLC) films on the polymer substrates via acetylene plasma, functionalization was done using ammonia plasma. The NH_3 plasma exposure time was varied from 0 to 300 s resulting in a different heparin coverage. For the different treatment times, heparin was successfully immobilized on the functionalized DLC leading to an extended blood coagulation time. The maximum of bound heparin was obtained at a 30 s treatment. Moreover, different heparin coverages were obtained for the different substrates. Based on these observations, the authors suggested that the initial surface roughness and the plasma treatment time i.e. the surface chemical structure influence the final heparin coverage.

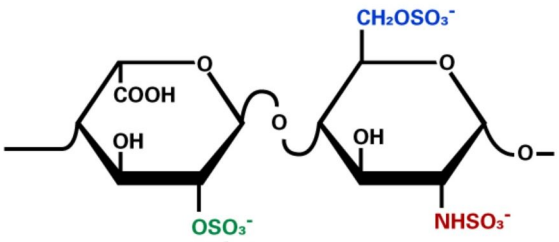

Figure 8. Chemical structure of heparin molecule [95]

In another important studied bio application related to biomedical implant devices, cell adhesion is very important because it is considered to be the determinant of the success or failure of implantation. Anchorage-dependent cells such as fibroblasts and osteoblasts need the adhesion for cell growth, division and spreading [79]. It was found that attachment, proliferation and function of these anchorage-dependent cells are highly dependent on the surface properties of biomaterials [80]. The variation of biocompatibility after amine plasma treatment of many biomaterials has been studied by the means of fibroblast and osteoblast cell cultures. Wen-Juan et al. [71] and Ren et al. [81] modified silicon surfaces with allylamine dielectric barrier discharge and microwave plasma respectively. After plasma exposure, the

contact angle decreased considerably due to the formation of various nitrogen functionalities as determined by FTIR. Cell culture tests with fibroblasts showed that both cell adhesion as well as cell proliferation could be improved by allylamine plasma treatment. Comparable results were obtained by Zelzer et al. [82] using glass substrates.

In a study by Hamerli et al. [43], the surface of polyethylene terephtalate (PET) was modified via allylamine microwave plasma polymerization. Plasma process parameters such as power (MW power), monomer flow rate ($Ø_{Allylamine}$) and duty cycle were varied which allowed the formation of different film chemical compositions. FTIR and XPS indicated that nitrogen as well as oxygen functionalities were incorporated which resulted in an increased hydrophilicity. Pictures from scanning electron microscopy showed that homogeneous pinhole-free allylamine plasma polymer (PPAa) films were obtained. Cell tests revealed improved cell attachment and spreading on PPAa coated PET compared to plain PET (see figure 9) with a greater improvement of biocompatibility on plasma polymerized allylamine coated surfaces containing amine functionalities in relatively high concentration. This is in agreement with other researches indicating that amino groups rather than others are favorable for protein adhesion [83, 84].

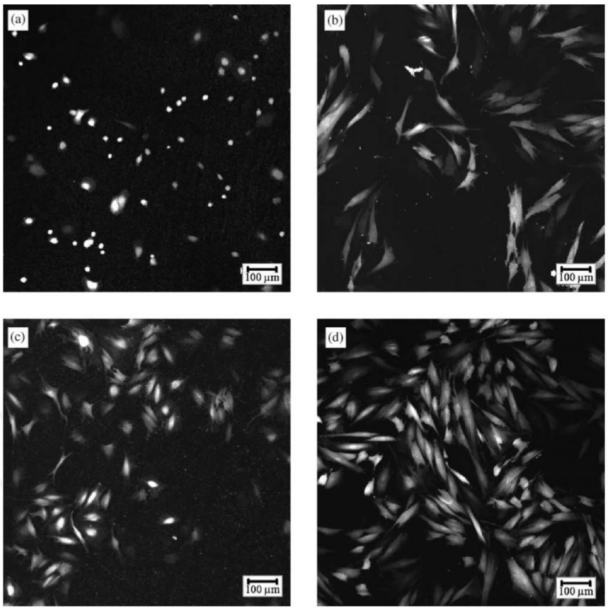

Figure 9. Photomicrographs of fibroblasts adhering on PET and PPAa membrane surfaces; (a,c) 4h of cultivation, (b,d) 24h of cultivation, (a,b) PET membrane, and (c,d) PPAa coated PET membrane "MW power of 1200W and $Ø_{Allylamine}$ of 30 sccm" [96]

In another study by Hamerli et al. [47], PET membranes were modified by ammonia and allylamine microwave plasma treatments. Both plasma treatments yielded an approximately

similar decrease in contact angle. Cell tests showed that fibroblast adhesion and spreading was improved for both plasma treatments compared to plain PET with higher proliferation on allylamine-modified samples in comparison to ammonia-plasma modified samples. This is mainly attributed to the higher amine concentration on allylamine modified PET.

Other studies involving osteoblast cell tests were also conducted on amine plasma polymerized films resulting in an improved cell adhesion and proliferation [39, 59, 65].

Substrate	Plasma media	Cell / biomolecule type	Observations	Ref
316L stainless steel	Allylamine + Ammonia + Ar	Heparin, endothelial cells	Good immobilization of heparin/ improved hemocompatibility/ promotion of adhesion and proliferation of endothelial cells	[77]
Silicon	Allylamine + Ar	fibroblast	Improved cell adhesion and growth	[81]
Silicon	1,2-diaminocyclo-hexane	Plasma proteins	Improved protein adsorption	[83]
Polysulfone	Ammonia/n-butylamine/ allylamine	Glucose isomerase	Successful immobilization of enzyme	[26]
BK7 glass	Allylamine	DNA	Successful immobilization of DNA	[85]
Polysiloxane	Allylamine	Spinal cord cells	Cell attachment and growth after 14 days culture	[86]
Silicon	Allylamine + Ar	Fibroblast	Promotion of cell growth and maintenance of their activity	[71]
Glass	Allylamine	Fibroblast	Improved cell adherence and proliferation	[82]
Polyethylene terephtalate	Allylamine + Ar	Fibroblast	Improved cell attachment and viability	[43]
Titanium alloy	Allylamine	Lysozyme and protein-4 (BMP-4)	Immobilization of biomolecules and retained activity on high amino groups density surfaces	[87]
Titanium	Allylamine	Monocyte, macrophage, T-	Minor reduction of inflammatory reactions in low water solubility films	[58]

Substrate	Plasma media	Cell / biomolecule type	Observations	Ref
		lymphocyte, MHC-class-II-positive cells		
Polyethylene terephtalate	Ammonia/ allylamine	Fibroblast	Improved cell adherence, spread and growth for both processes with greater improvements for the plasma polymerization process	[47]
Polytetra-fluoroethylene, polystyrene and silicone	Acetylene then ammonia	Heparin	Good immobilization of heparin and retarded blood coagulation	[78]
Polyethylene terephtalate and polytetra-fluoroethylene	Ammonia	Human umbilical vein endothelial cells	Improved cell adhesion and growth	[88]
Polyethylene terephtalate	Allylamine	Fibrinogen	Increased protein adsorption and denaturation	[89]
Titanium	Allylamine + Ar	Osteoblast	Improved initial cell adhesion and spreading	[39, 65]
Titanium alloy	Allylamine / ethylene-diamine / nylon + N_2,H_2	Osteoblast	Improved initial cell adhesion and spreading	[59]
Si wafer	Propylamine/ allylamine/ propargyl-amine	Fibroblast	Improvement of cell attachment and proliferation with better results for PPA than PPP and PPG	[60]

Table 1. Representative overview of plasma processes for the incorporation of amine groups and their influence on cell-material interactions

6. Conclusions and future trends

From the work presented in this chapter, it is clear that amine plasma based techniques are powerful versatile tools to modify a material surface in order to make it more biocompatible. However, despite the vast array of choices offered by plasma processing techniques, there remain a significant number of hurdles that must be overcome to allow this methodology to realize its full potential in the area of biocompatible materials. Challenges include issues associated with analytical characterization, material structure, plasma processing, and uniform composition and thickness following treatment. A better understanding of the cell-biomaterial interactions is of crucial importance providing us with essential clues about which strategies should be pursued. As our fundamental understanding of these complex phenomena further develops, better control over cell adhesion, proliferation and differentiation will

be achievable. Up till now, plasma surface modification is mostly studied on two-dimensional substrates but it can be expected that the study of 3D porous scaffolds will become more important. It is believed that, in the future, a broader exploration of various plasma strategies, monomers and biomaterials will enable us to achieve homogeneous scaffolds with desired mechanical, chemical and biodegradation properties in an industrialized setting.

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